REPRODUCTIVE BIOLOGY OF THE CARRION-FLOWER, *SMILAX HERBACEA* (SMILACACEAE)

Neil W. Sawyer and Gregory J. Anderson

The University of Connecticut,
Department of Ecology and Evolutionary Biology,
75 North Eagleville Road, Box U-43, Storrs, CT 06249-3043

ABSTRACT.   Pollination biology, reproductive output, and population structure of male and female plants and flowers of *Smilax herbacea* were studied in nearly 900 individuals from 11 roadside populations in northeast-central Connecticut over a three-year period. Percentages of either sex did not vary across sites or between years. Reproductive plants represented 60% of an average population and nearly 65% of these were male. Female mortality is suggested as the most likely reason for the strong male bias (2.3♂:1♀). The male plants, although smaller, had twice the number of inflorescences, and 68% more flowers than female plants. Both inflorescence and flower number were significantly correlated with plant size in male plants. This correlation was absent for female plants implying that male plants are less constrained in the number of inflorescences and flowers they can produce. Although site size, population size, and population density of both female and male plants varied significantly across sites, gender and overall plant densities were not correlated with site size. However, a significant interaction was found between the densities of each sex and the site of their occurrence, suggesting that distribution of gender patterns may be influenced by site-specific ecological factors. *Smilax herbacea* can be characterized as a generalist in its attraction of pollinators. The carrion-like odor produced in tepals of male and female flowers, and the pollen reward of the staminate flowers attract bees, beetles, and flies. Due primarily to their high mobility and good pollen carrying capacity, andrenid and halictid bees and anthomyiid and stratiomyiid flies are considered major pollinators. Minor pollinators include soldier (Cantharidae), long-horned (Cerambycidae), and fire-colored (Pyrochroidae) beetles. Overall fruit set was lower in 1995 than in 1994 or 1996; this likely was due to unusually wet conditions that year. No significant difference in fruit set among populations was detected. Less than 1% of intrapopulational fruit set variation was explained by either site size or by plant density. Male plant densities were not significantly correlated with fruit set across sites. Furthermore, experimental interpopulational crosses did not significantly increase fruit set. These facts imply resource rather than pollen limitation in female plants.

Key Words: *Smilax herbacea,* dioecy, sex ratio, pollination mechanisms, entomophily, fruit set, pollen limitation, resource limitation

The genus *Smilax* L. comprises about 200 tropical and tem-

perate species all of which are dioecious lianas, scramblers, or herbs. Species of *Smilax* have long been recognized as insect pollinated (Delpino 1880), and were grouped by Lovell (1920) with green flowered species pollinated by flies, beetles, and small bees. Honeybee visitation to some species was observed by Pellet (1976). For *S. rotundifolia,* Kevan et al. (1991) recorded visits from a *Bombus* sp. and mosquitoes. Nothing further has been reported regarding the pollination biology in the genus. This study was undertaken to better understand the reproductive biology and the mechanisms of pollination in dioecious *S. herbacea,* and to test hypotheses regarding the adaptive significance of flower form and function.

Smilax herbacea L., distributed from southeastern Canada south to Georgia and west to Manitoba and Missouri, is an herbaceous, perennial geophyte sprouting an annual shoot system from a short, thickened rhizome. Plants occur most commonly along roadsides, but also in meadows, at forest edges, or within forests. The shoot system may be branched, and may extend to a length of close to three meters. Plants remain erect for at least a portion of their length, then become scandent. Shoots attach to neighboring vegetation by paired, stipular tendrils. This species is dioecious. It has been suggested that sex in *S. herbacea* is determined by X and Y chromosomes (Mangaly 1968) among the $n = 13$ chromosomes (Lindsay 1930). Plants of *S. herbacea* flower in Connecticut during the last week in May through the first three weeks in June, the fruit maturing in September. Inflorescences occur as orbicular, pedicellate umbels on long, axillary peduncles either on primary axes or, less often, on lateral branches (Figure 1). The inflorescences are conspicuous and odoriferous. Flowers average 8–10 mm in diameter and bear six green tepals with glandular papillae along their margins (Pennell 1916). Further, the common name is appropriate, and the carrion-like odor released by the flowers of *S. herbacea* led us to suspect sapro-cantharophily or sapromyophily (Faegri and van der Pijl 1979). Staminate flowers bear six stamens with undifferentiated, bilocular anthers. Both the anthers and their copious pollen are white to yellow-white. Pistillate flowers bear conspicuous, green ovaries with greenish-white stigma lobes. Filament remnants are obvious at the base of the ovary. The advertising of a pollen reward by the yellowish-white anthers in males is mimicked in female flowers by the relatively large stigma lobes.
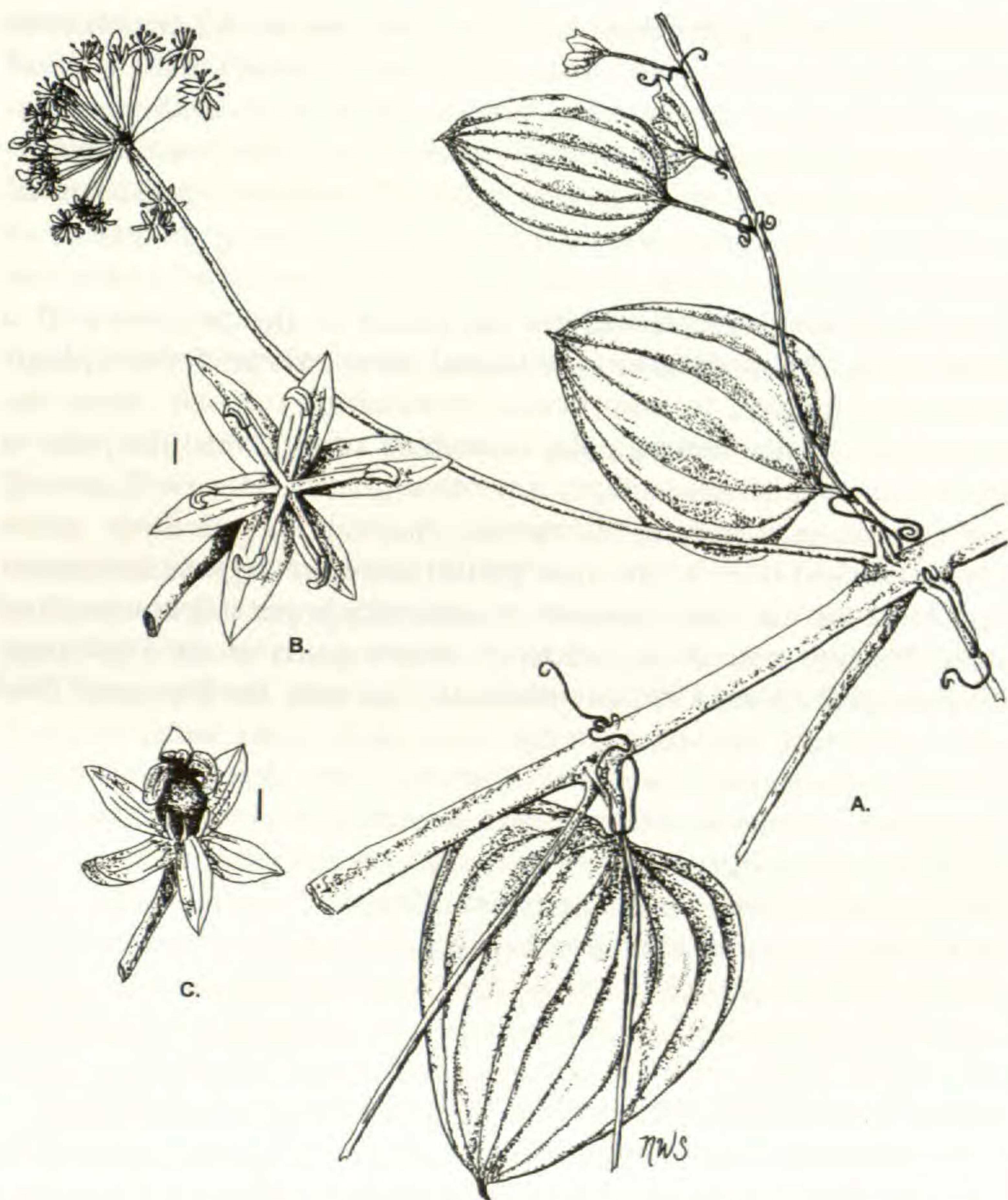
Figure 1. *Smilax herbacea*. A. Stem segment of mature male plant—9/16 actual size. B. Staminate flower. C. Pistillate flower. Bars = 1 mm.

MATERIALS AND METHODS

Population demographics. Eight hundred sixty-nine individual plants were observed in 11 roadside populations in northeast-central Connecticut from May through June and from late August through September during 1994 and 1995. Additionally, fruit set was assayed in 1996. Representative vouchers are deposited in the University of Connecticut herbarium (CONN). Roadside populations were selected because they were the only sites

where flowering individuals occurred. For this study, populations were defined as localized concentrations of plants with at least one female plant present. The plants within each of the populations were never more than nine meters from their nearest neighbor. The presence of a female plant allowed intrapopulational crosses and interpopulational fruit set comparisons. Plants in all populations grew within three meters of a road. Site perimeters were arbitrarily determined by the plants at the periphery of a population. All populations bordered areas where *Smilax* plants were absent, viz., forests, fields, or dwellings. In all cases, the peripheral plants defining the boundary of a given site were a minimum of 500 meters from any other plants. Because flowering individuals occurred in disturbed, roadside populations, some plants marked over a two-year period were lost to summer mowing. Data for site size, number of individuals per population, and plant density were compiled from observations of six 1994 populations and of ten 1995 populations, five new for that year. One 1994 site was lost to mowing. Individual sites were mapped, plants were counted, and site size (m^2) was determined. Using these data, plant densities (plants per 1000 m^2) were calculated. Population demographics were further investigated by recording the number of individuals per population and scoring individuals as female, male, or nonreproductive. Because *S. herbacea* is not clonal, individuals were easily identified. Sex ratios were determined and compared among populations using the G-test (Sokal and Rohlf 1995). The size of male and female plants was determined by measuring the length of primary stems at anthesis.

To investigate the relationships between site size and plant density (all individuals, or males, or females) and between site size and reproductive output (mature fruit set), tests for correlations were conducted. Tests for correlations were also conducted between fruit set per site and plant density per site. To determine whether the density of pollen donors within a site had an effect on fruit set, a correlation was looked for between mean fruit set and the density of male plants across sites. To determine if sex expression was independent of site, a Chi-square test of independence was performed using the frequencies of male, female, and nonreproductive plants for each of the ten 1995 sites.

Staminate and pistillate flower demographics, morphology, and phenology. The number of inflorescences per plant was

determined for all members of each population. The number of flowers per inflorescence was determined from randomly selected male and female plants from each population. Relationships between either flower number or inflorescence number and the size of the plants, measured as the length of primary shoots, were investigated by regressing flower number on inflorescence number and inflorescence and flower number on plant size.

The morphology of staminate and pistillate flowers was investigated and compared across populations. Both dried and alcohol-preserved voucher specimens were collected. Perianth diameters of 50 pistillate flowers from five populations and 50 staminate flowers from six populations were measured in the laboratory using alcohol-preserved specimens. These measurements were compared using a two-way ANOVA.

Study of flowering phenology involved random selection and marking with acrylic paint of 55 staminate flowers and 52 pistillate flowers from among 30 inflorescences (from which insects were excluded), three from each 1995 population. The maturation of marked flowers was recorded twice each day, early morning and late afternoon, until senescence. Stigmas were considered receptive while color, size, and appearance remained constant. Loss of receptivity was indicated by a darkening of the stigmatic surface accompanied by shrinkage of the stigma lobes and/or loss of tepals. Longitudinal sections of fresh flowers were made to determine the presence or absence of nectaries.

Pollination biology. Faegri and van der Pijl (1979) established criteria that allow discrimination of pollinators from scavengers. First, a relationship must exist between the flower and the pollinator that contributes to regular pollination, i.e., an attractant or reward must be present. Second, the body of the pollinator should mechanically promote pollen adherence allowing for effective pollen dispersal. Finally, the pollinator should demonstrate a high frequency of flower visits, thus requiring at least moderate mobility. Baker et al. (1971) suggested that major and minor pollinators might be distinguished by their degree of mobility, assuming more pollen will be moved around by more mobile visitors. Floral visitors were evaluated as potential pollinators based on these four criteria. In order to obtain a quantitative estimate of pollinator activity, 50 inflorescences, 25 for each sex, from seven populations were observed and assessed during 10-minute

intervals for the frequency of visitation and movement patterns. Insect visitors were surveyed for the presence or absence of pollen grains. If pollen was present, a pollen sample was removed and examined for grains of *Smilax herbacea*. Flower visitors were either collected for identification or noted (when identity known) whenever they were observed on flowers. Voucher specimens of all insect visitors have been deposited in the University of Connecticut insect collection.

Reproductive output. The number of fruits matured per infructescence and the percent fruit set were determined for randomly selected infructescences during 1994 and 1995. Because fruit set data for the 1995 season displayed adverse effects, presumably caused by unusually wet weather conditions, 1996 fruit set data from 12 populations were included. Three populations were new for that year. Pedicel scars on the infructescence axis gave a precise indication of the number of flowers produced; thus, it was not necessary to count flowers prior to counting fruits to determine fruit set. To account for possible loss to birds, green fruits were counted and subsequently followed to maturity. In 1994, fruit weight, the number of seeds per fruit, and weights of seeds were determined by sampling ripe fruit. Weights and counts of seeds were determined by separating the seeds from the fruit, counting the number of seeds per fruit, cleaning the seed by hand, air drying the seeds at ambient temperatures for one week, recleaning, and weighing.

Crosses were performed artificially by touching the pollen-containing anthers of staminate flowers to the stigmatic surface of pistillate flowers in inflorescences that had been bagged prior to hand pollination. All bags used in these experiments were constructed from white, nylon insect netting with a 1 mm × 0.75 mm mesh. To ensure stigmas were receptive, pollinations were performed within 48 hours of flower opening. Fruit set resulting from these crosses was compared with that of controls. Both interpopulation and intrapopulation crosses were performed. Analyses of variance tests were performed within and among populations to detect patterns of variation in fruit set.

RESULTS

Population demographics. Site size varied greatly, from 50 to 1045 m² (Table 1). Populations consisted of from 16 to 254

Table 1. Population size, numbers of individuals by sex, and sex ratios for eleven 1994 and 1995 sites. * $P < 0.5$, ** $P < 0.01$.

| Population	Size (m²)	Year	Number of Individuals	Males #	Males %	Females #	Females %	Nonflowering #	Nonflowering %	Hermaphrodites #	Hermaphrodites %	Ratio	G Statistics
Bolton Road	450	1994	—	—	—	—	—	—	—	—	—	—	—
		1995	30	14	46.7%	6	20%	10	33.3%	—	—	2.3:1	0.00
Cedar Swamp Road	550	1994	—	—	—	—	—	—	—	—	—	—	—
		1995	254	131	51.6%	39	15.3%	84	33.1%	—	—	3.5:1	4.25*
Bone Mill Road	225	1994	26	6	23.1%	5	19.2%	15	57.7%	—	—	1.2:1	1.16
		1995	16	5	31.3%	7	43.7%	4	25%	—	—	0.7:1	4.12*
South Eagleville Road	170	1994	—	—	—	—	—	—	—	—	—	—	—
		1995	41	16	39%	13	31.7%	11	26.8%	1	2.5%	1.2:1	2.83
Agronomy Farm	165	1994	52	27	51.9%	8	15.4%	17	32.7%	—	—	3.5:1	0.90
		1995	54	33	61.1%	3	5.6%	18	33.3%	—	—	10.2:1	10.11**
Knowlton Hill Road	1045	1994	—	—	—	—	—	—	—	—	—	—	—
		1995	134	52	38.8%	34	25.4%	47	35.1%	1	0.7%	1.6:1	3.54
Wormwood Site	185	1994	—	—	—	—	—	—	—	—	—	—	—
		1995	58	16	27.6%	3	5.2%	39	67.2%	—	—	5.6:1	2.06
Daleville Road North	185	1994	39	17	43.6%	10	25.6%	12	30.8%	—	—	1.7:1	0.61
		1995	46	20	43.5%	10	21.7%	16	34.8%	—	—	1.9:1	0.16
Daleville Road South	50	1994	26	14	53.8%	6	23.1%	6	23.1%	—	—	2.3:1	0.00
		1995	28	4	14.3%	5	17.8%	19	67.9%	—	—	0.8:1	2.53
Cary Tract	210	1994	23	12	52.2%	7	30.4%	4	17.4%	—	—	1.7:1	0.41
		1995	23	8	34.8%	5	21.7%	10	43.5%	—	—	1.6:1	0.42
Gurleyville Road	120	1994	19	6	31.6%	3	15.8%	10	52.6%	—	—	2:1	0.05
		1995	—	—	—	—	—	—	—	—	—	—	—
All sites	3355	1994	185	82	44.3%	39	21.1%	64	34.6%	—	—	2.1:1	—
		1995	684	299	43.7%	125	18.3%	258	37.7%	2	0.3%	2.4:1	—
		Both years	869	381	43.8%	164	18.9%	322	37.1%	2	0.2%	2.3:1	14.66

■ Males ▨ Females ☐ Nonreproductive
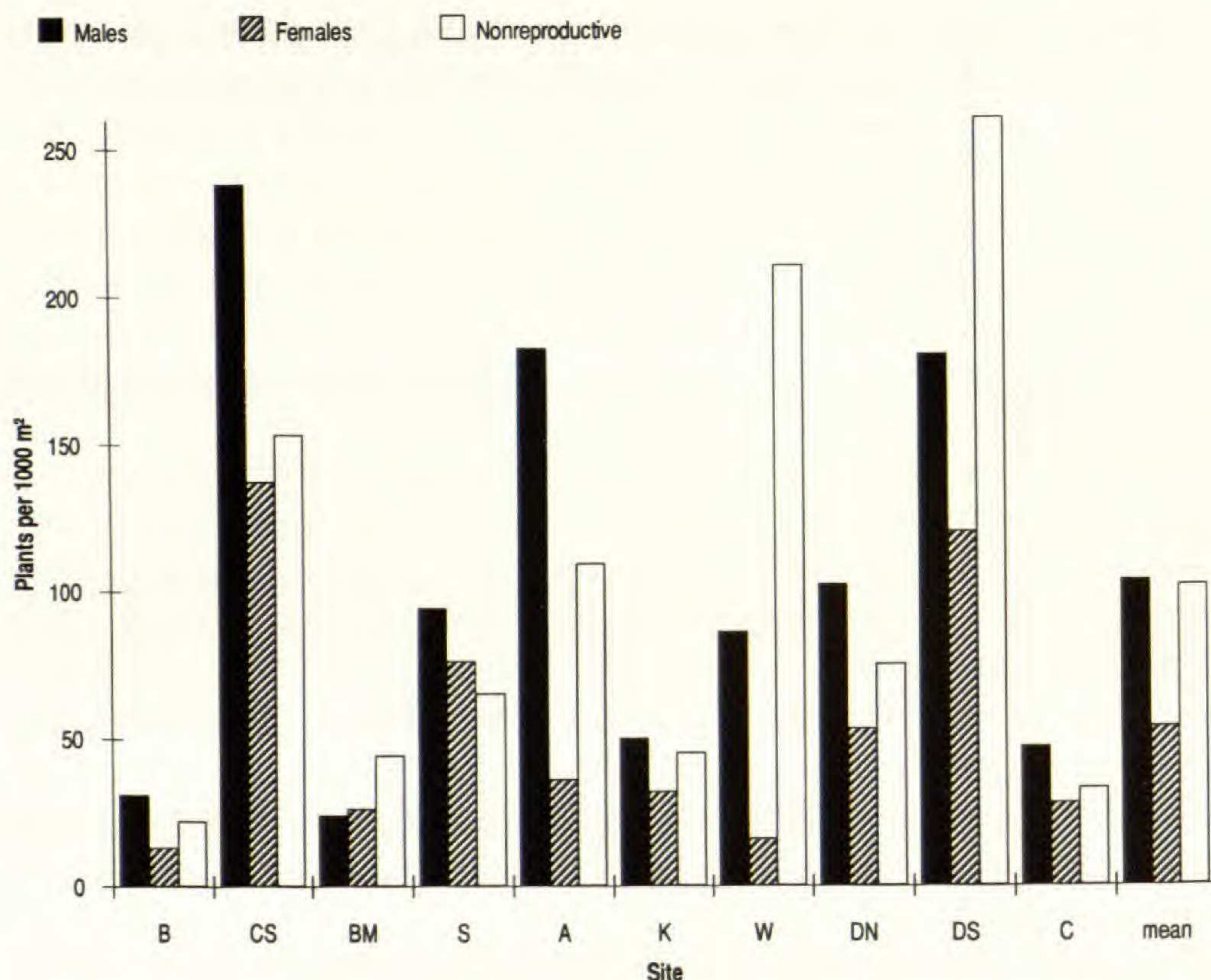
Figure 2. Comparison of mean population densities (plants per 1000 m^2) for male, female, and nonreproductive plants in ten 1995 populations. (B = Bolton Rd., CS = Cedar Swamp Rd., BM = Bone Mill Rd., S = S. Eagleville Rd., A = Agronomy Farm, K = Knowlton Hill Rd., W = Wormwood Site, DN = Daleville N. Rd., DS = Daleville S. Rd., C = Cary Tract)

individuals (mean per population = 54; Table 1). Staminate and pistillate plant densities also varied greatly from site to site (Figure 2). Neither male, female, nor overall plant densities were significantly correlated with site size (♂, r = 0.040, n = 10; ♀, r = 0.060, n = 10; overall, r = 0.305, n = 10, respectively). However, a Chi-square test for independence using frequencies of male, female, and nonreproductive plants across all ten 1995 populations showed a highly significant interaction between individual sites and gender densities among sites (X^2 = 250.88, 18 df, P < 0.001).

The mean percentages of plants of each sex from all sites for both years were as follows: ♂'s = 44%, ♀'s = 19% (Table 1). This gives a male biased sex ratio of 2.3♂:1♀. Significant heterogeneity in sex ratios was detected among sites, and three sites varied significantly from the 2.3♂:1♀ ratio. Further, although in-

Table 2. Mean number of inflorescences per plant among reproductive individuals in eleven 1994 and 1995 populations.

Population	Year	Males	Females	Hermaph-rodites
Bolton Road	1995	12.1	2.8	—
Cedar Swamp Road	1995	14.4	6	—
Bone Mill Road	1994	29.4	6.4	—
	1995	26	17.8	—
South Eagleville Road	1995	22.7	4.9	14
Agronomy Farm	1994	14	7.1	—
	1995	18.6	12.3	—
Knowlton Hill Road	1995	19.3	5	5
Wormwood Site	1995	9.1	5.3	—
Daleville Road North	1994	15.3	10.5	—
	1995	16.9	5.9	—
Daleville Road South	1994	10.1	3.2	—
	1995	4.5	2	—
Cary Tract	1994	10	4.1	—
	1995	11	5	—
Gurleyville Road	1994	7	7	—
All sites	1994	14.8	6.4	—
	1995	15.5	6.7	9.5
	Both years	15.1	6.5	4.7

dividual populations showed marked differences in sex ratios between years, an analysis of variance performed on arcsine transformed data showed that neither the percentage of males nor the percentage of females varied significantly between years across all populations ($F = 0.328$, $df = 15$, $P = 0.576$, for males; $F = 0.107$, $df = 15$, $P = 0.748$, for females).

The mean length of the primary stems was 109 cm for male plants and 164 cm for female plants. Analysis of variance indicated that these differences were significant ($F = 8.12$, $df = 32$, $P < 0.01$).

Staminate and pistillate flower demographics, morphology, and phenology. Plants of both sexes varied greatly in the number of both inflorescences and flowers. The mean number of inflorescences per staminate plant was 15 (Table 2; range = 1–60), and each inflorescence bore an average of 30 flowers (range = 4–100). This is contrasted with female plants that averaged 6.5 inflorescences per plant (Table 2; range = 2–28) and averaged 41 flowers per inflorescence (range = 12–110). When all sites

were considered for both years, male plants bore significantly more inflorescences than female plants (F = 19.77, df = 31, P < 0.001). When both flower number and inflorescence number were considered, male plants (average of 590 flowers/plant; Table 3) produced 68% more flowers than female plants (average of 351 flowers/plant; Table 3). Males showed greater variation in the number of inflorescences produced per plant than females (r^2 = 44.5, n = 214 for ♂'s; r^2 = 31.8, n = 116 for ♀'s). Flower numbers per inflorescence also varied greatly in both sexes (Table 3). Flower number per inflorescence consistently decreased with distance from the base along the primary axis. This is reflected in a highly significant negative correlation between the number of flowers in an inflorescence and the distance in cm of that inflorescence from the base (r = 0.957, n = 29, P < 0.001). Plants producing fewer inflorescences were likely to exhibit relatively less variation in flower number per inflorescence. Consequently, variation in flower number per inflorescence was more likely to be detected in plants producing many inflorescences, i.e., in male plants.

Little of the variation in either inflorescence or flower number was explained by plant height when plants were compared without regard to sex (r^2 = 0.118, P = 0.0628; r^2 = 0.2182, P = 0.0053 for inflorescence and flower number, respectively). However, when male and female plants were considered separately, much of the variation both in flower number (r^2 = 0.755, P = 0.0014) and inflorescence number (r^2 = 0.6858, P < 0.0001) was explained by plant height in male plants, whereas very little of the variation either in flower number (r^2 = 0.009, P = 0.3113) or inflorescence number (r^2 = 0.0947, P = 0.3573) was explained by plant height in female plants.

The diameter of the pistillate perianth averaged 8.0 mm (range = 5.9–9.3 mm). The mean diameter of staminate perianths was 10.1 mm (range = 6.9–12.5 mm). Although staminate flowers tended to be larger in diameter than pistillate flowers, a two-way ANOVA detected neither a significant difference in the variance between the sexes (F = 1.081, df = 1, P > 0.75) nor an added variance component due to population (F = 0.053, df = 4, P > 0.75).

Male plants typically inaugurated the reproductive season by flowering one to several days earlier than female plants. Many other differences were observed between staminate and pistillate

Table 3. Individual samples of plant height and flower production from six 1994 populations. * Numbers identify plants in specific populations: G = Gurleyville Road, C = Cary Tract, DS = Daleville Road South, DN = Daleville Road North, A = Agronomy Farm, B = Bone Mill Road.

Plant ID*	Height (cm)	Flowers/ Inflorescence	Inflorescences/ Plant	Total Number of Flowers/Plant
Female Plants				
G5	186	43	10	430
C12	237	42.1	7	295
C17	198	28.7	6	172
DS12	74	24.5	2	49
DS17	121	22.7	3	68
DS18	142	46.6	5	233
DS20	115	14	3	42
DN11	250	68.3	6	410
DN12	271	33	4	132
DN13	274	43.8	6	263
DN14	274	42	5	210
A37	236	68.2	27	1841
B1	173	66.3	10	663
B15	167	25.5	4	102
Mean	194	40.6	7.0	351
Male plants				
G3	144	42.8	23	939
G7	77	16.8	6	101
G8	75	7	5	35
G14	86	27.7	13	333
C5	167	24.6	5	123
C11	148	26.8	20	536
DS10	73	24	4	96
DS14	101	21.4	13	278
DS15	147	42.4	32	1357
DS19	88	28.5	4	114
DN5	157	33.5	24	805
DN17	141	25.3	12	304
DN23	244	45.2	34	1537
A13	100	14.4	7	101
A17	102	16.3	14	228
A20	67	16.2	5	81
A23	205	38.1	30	1144
B6	124	42.5	13	552
B12	175	48.1	14	673
B17	265	61.6	40	2465
Mean	134	30.2	15.9	590

flower maturation and between patterns of pollen availability and stigma receptivity. Staminate flowers consistently matured in a centripetal pattern, whereas flowers in female inflorescences did not exhibit any recognizable maturation pattern. Anthers dehisced well after the odor-producing tepals had reflexed, whereas stigmas were receptive the moment pistillate flowers opened. Due to pollen collection by pollinators and continual pollen predation by beetles, pollen was available in any given staminate flower for about one day, whereas pistillate flowers had receptive stigmas for three to four days. When simultaneously flowering staminate vs. pistillate flowers were compared, stigma receptivity consistently outlasted the pollen release of anthers. In addition, within an inflorescence many more pistillate flowers matured per day than did staminate flowers. When the overall means of individual staminate and pistillate inflorescence life spans (life span measured as the duration of pollen availability in a given staminate inflorescence, or the duration of stigma receptivity in a given pistillate inflorescence) were compared, no significant difference was detected (mean = 6.6 days for 114 staminate inflorescences [3138 flowers] in four plants; mean = 6.9 days for 38 pistillate inflorescences [2222 flowers] in four plants; $t = 2.447$, $P > 0.2$).

Pollination. Tepals in both male and female flowers of *Smilax herbacea* are green and were observed to secrete a liquid through glandular, epidermal papillae along the margins and at the apices of the tepals. This liquid is ignored by insect visitors. These compounds presumably are those responsible for the carrion-like odor, for when the tepals are removed, no odor is apparent. Based on observations of sectioned ovaries, there is no anatomical evidence for nectar production.

The pollen grains of *Smilax herbacea* do not contain viscin threads as Kevan et al. (1991) reported for *S. rotundifolia*, but are distinctly spinose with a very sticky pollenkitt. Pollen was moved from male to female flowers by insects. Of all the observed floral visitors, 89% were coleopteran, 4% were hymenopteran, and 7% were dipteran (Table 4). False darkling beetles (Scraptiidae, genus *Anaspis*) constituted 74% of the individual observations among coleopteran visitors and 66% of all visitors. These tiny beetles, however, lack hairs or other means of pollen dispersal and are sedentary, breeding on both female and male flowers and only occasionally feeding on the pollen of male flow-

ers. Soldier beetles (Cantharidae) accounted for 13% of all beetles observed, long-horned beetles (Cerambycidae) for 5%, and fire-colored beetles (Pyrochroidae) for 4%. Species in these three families are moderately mobile (see below) and possess hairs and body parts to which pollen adheres. Click beetles (Elateridae) accounted for the remaining 1% of beetle observations. Click beetles have smooth body parts and lack hairs. No pollen was found on these beetles. Hymenopteran visitors consisted of two species of halictid bees and one species of andrenid bee. All these bees have hairy bodies, and large amounts of pollen were present on all individuals. These bees are also highly mobile. Of the four families of dipteran visitors, only the small scavenger flies (Anthomyiidae) and the large soldier flies (Stratiomyiidae) were found to carry pollen (Table 4). Both of these flies were highly mobile, possessing hairs on their legs and bodies among which large amounts of pollen were found. However, these flies accounted for only 1% of all observations.

Using the criteria given in the methods, floral visitors were evaluated as possible effective pollinators: beetles accounted for 80% of the possible pollinator frequency, bees for 13%, and flies for 7% (Table 4). Although accounting for only 20% of possible pollinators, small halictid and andrenid bees and anthomyiid and stratiomyiid flies were observed to be strikingly more mobile when contrasted with the relatively sedentary beetles. Insect visits were recorded for 20 of the 25 male inflorescences observed, but for only seven of the 25 female inflorescences. Beetles carrying *Smilax herbacea* pollen (cantharids, cerambycids, pyrochroids) were nearly immobile, spending at least 75 seconds on a single flower and often much longer. Further, beetles remained within the same inflorescence during the entirety of each 10 minute observation interval (Figure 3). Beetles were seldom observed visiting female inflorescences. Bees and flies, on the other hand, spent 16 seconds or less (flies = 16 seconds maximum, bees = 9 seconds maximum) on a single flower and 32 seconds or less (flies = 32 seconds maximum, bees = 19 seconds maximum) within a single inflorescence (Figure 3). Without exception, bees and flies visited many more flowers and plants of both sexes than beetles within each observation interval. Individual flies visited a minimum of nine flowers on four inflorescences of a single plant during an 82-second interval before moving to another plant. Individual bees visited a minimum of 10 flowers on six inflores-

Table 4. Frequency of insect visitors based on results of 27 ten-minute observation intervals. Families in bold have members considered likely pollinators. [a] The frequency of each visitor is expressed as the number and percentage of all observations.

Order	Family	Genus	Frequency of All Visitors[a]	Pollen Carriers (% of all individuals)
Coleoptera				80%
	Scraptiidae			
		Anaspis	160 66%	—
	Cantharidae			
		Podabrus	31 13%	46%
		Cantharis	1 —	1%
	Cerambycidae			
		Anoplodera	12 5%	18%
	Pyrochroidae			
		Pedilus	10 4%	15%
	Elateridae		2 1%	—
Hymenoptera				13%
	Halictidae		7 3%	10%
	Andrenidae		2 1%	3%
Diptera				7%
	Anthomyiidae		3 1%	4%
	Muscidae		12 5%	—
	Stratiomyiidae		2 1%	3%

cences on the same plant during a 52-second interval before moving to another plant. No differences were detected in the timing of bee and fly visitations between male and female flowers or inflorescences.

Reproductive output. All inflorescences (n = 10) that remained bagged for the duration of flowering showed 0% fruit set. Overall matured fruit set in open-pollinated controls, although quite variable, was lower in 1995 (53% from 9 sites) than in 1994 (69% from 6 sites; Table 5) or 1996 (73% from 12 sites; Table 5). The 1995 season was unusually wet and many infructescences succumbed to mildew, thus lowering fruit set potential for that year. Analyses of variance performed on arcsine transformed data revealed significant differences between fruit set in 1995 and both 1994 (F = 4.96, df = 13, P < 0.05) and 1996 (F = 10.39, df = 20, P < 0.005). Analysis of variance detected no significant dif-
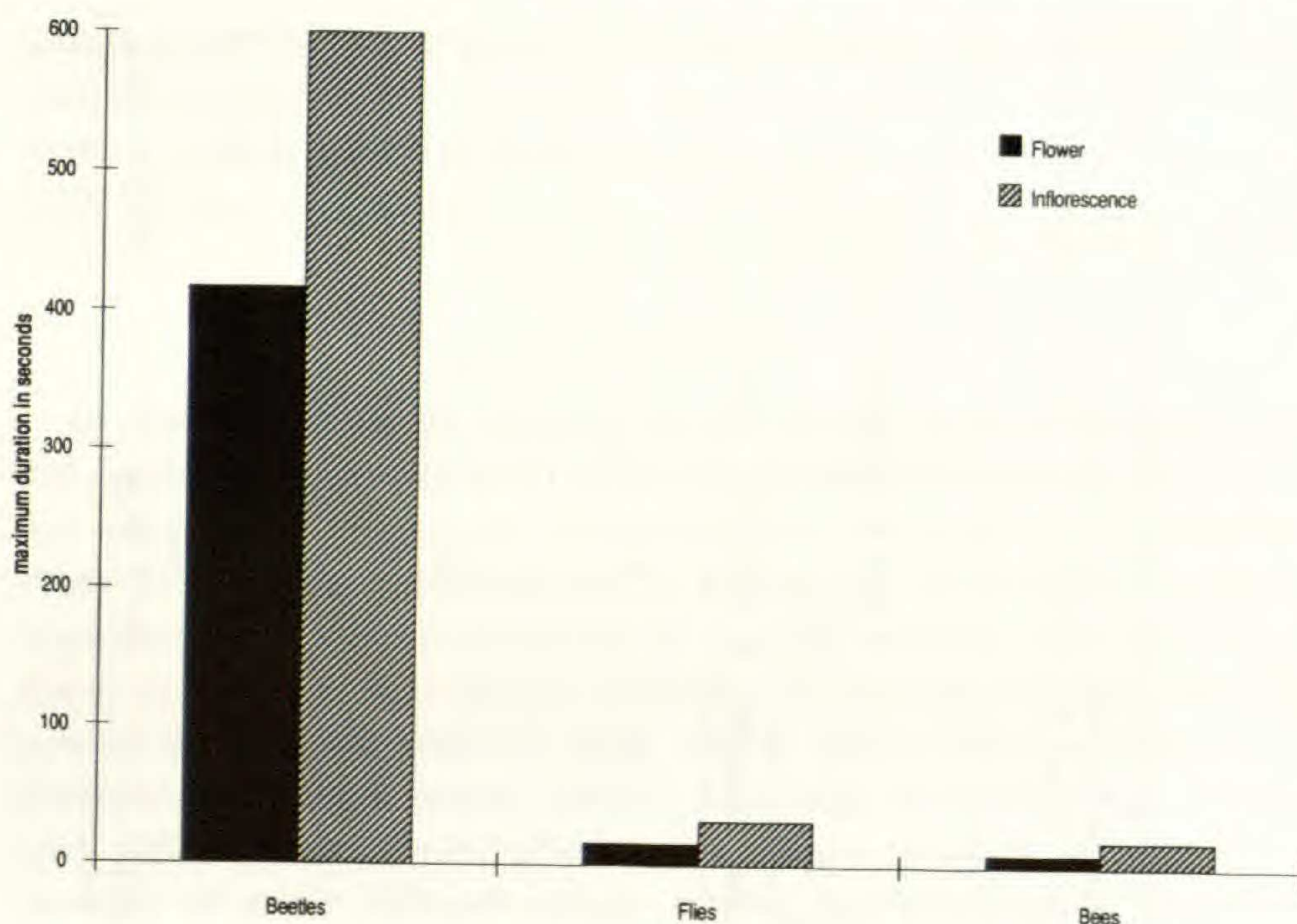
Figure 3.   Comparison of the maximum observed duration of insect visits to flowers and inflorescences based on 27 ten-minute observation intervals (1994).

ference in fruit set between 1994 and 1996 (F = 0.037, df = 16, P = 0.849). A two-level nested Model II ANOVA performed on arcsine transformed fruit set data among individuals from three 1996 populations showed neither significant variance among populations nor an added variance component due to individuals. No correlations were found between fruit set within a population and either the size of the population or the overall plant density of the population (r = 0.024, n = 9; r = 0.016, n = 9, respectively). Nor was a significant correlation found between male plant densities and mean fruit set across populations (r = 0.139, n = 9).

Hand pollination experiments showed higher fruit set for 1996 ($\bar{x}$ = 80%, n = 28; Table 5) than for 1995 (64%, n = 8; Table 5). Analysis of variance on arcsine transformed data showed that fruit set variance for the 1996 hand pollinations was not significantly different from the controls (F = 1.913, df = 19, P = 0.18). However, significant differences were detected in fruit set variance between the intrapopulation and interpopulation crosses for 1996 (F = 5.892, df = 13, P < 0.05).

Berries averaged 9 mm in diameter when mature. Fruit ranged in weight from 0.07 to 0.68 g averaging 0.36 g. The mean seed

Table 5. Fruit set data and number of infructescences sampled for five 1995 and nine 1996 interpopulation crosses, five 1996 intrapopulation crosses, and open pollinated controls. * Populations represented as in Table 3 with the addition of BO = Bolton Road, CS = Cedar Swamp Road, S = Silver Lane, BR = Brewster Road, NR = North River Road, SE = South Eagleville Road, K = Knowlton Hill Road, W = Wormwood Site.

Popula-	Hand Pollination						Open Pollination Controls			
tion*	Interpopulation Crosses				Intrapopulation Crosses 1996		1995		1996	
	1995		1996							
	Sample	% Fruit Set	Sample	% Fruit Set	Sample	% Fruit Set	Sample	% Fruit Set	Sample	% Fruit Set
BO			3	97			9	43	4	67
CS	2	11			5	68			12	70
S			3	66					14	92
BR					1	45			7	85
NR			2	100	3	52			8	72
B	1	82	4	66			4	35	13	58
SE			3	51			2	87	1	74
A			1	78	2	80	5	39	4	55
K	1	100	7	74	4	25	11	56	17	100
W	3	52	2	95			8	57	3	80
C			3	99			3	40	4	53
DN	1	76					5	37		
Mean		64.2 ± 34.3		80.7 ± 17.8		54 ± 21.2		51 ± 18.0		73.3 ± 15.1

number per fruit was three (156 fruits from 6 sites in 1994). Seed weight ranged from 19 to 77 mg, averaging 32 mg (818 fruits from 6 sites in 1994). Seed weight typically accounted for about 25% of the fruit weight.

DISCUSSION

In *Smilax herbacea,* synchrony in pollen availability and stigma receptivity is critical to effective pollination and, therefore, to the success of dioecy as a reproductive strategy in this species. How is this accomplished in *S. herbacea*? Reproductive plants represent 60% of a population in a given season. Nearly 65% of these are male. Male plants typically inaugurate the reproductive season by one to several days, as in some other species (e.g., *Lindera benzoin*; Carloni 1982). In plants of both sexes, flower and inflorescence maturation on a single plant is staggered. In flowers of male inflorescences, pollen is dispensed in an incremental, timed-release pattern (i.e., all flowers do not open simultaneously), and, thus, pollen availability is extended over several days. Among inflorescences, pollen likewise becomes available gradually. The flowers of the basal inflorescences mature first, followed by incremental pollen release distally up the stem axis, resulting in overlap of pollen availability among inflorescences. Consequently, pollen of a single plant is available for up to several weeks. Because there is heterogeneity among males in inflorescence size and number as well as in temporal flowering patterns, pollen availability also overlaps within and among populations. Stigma receptivity in an individual flower lasts approximately three or four days and, as in males, there is incremental flower and inflorescence maturation. Therefore, we see that, with regard to flower production, the reproductive life spans of staminate and pistillate inflorescences are similar. However, because female plants typically have fewer inflorescences than male plants, the reproductive life span of a female plant will necessarily be shorter than that of a male plant. The more or less continual overlap of pollen production among male plants thus allows co-occurrence of pollen availability with stigma receptivity.

The overlapping of stigma receptivity by pollen production in plants of *Smilax herbacea* also can be considered in the context of the differential energetic costs of pollen production vs. the combined costs of ovule, seed, and fruit production. Greater re-

productive costs are associated with female plants than with male plants of dioecious species (e.g., Cipollini and Whigham 1994; Nicotra 1996). This phenomenon may be reflected in the number of inflorescences and flowers produced by individuals of each sex and by cost in relation to plant size. It might be expected that more vigorous plants, either because they are more fit or because more resources are available to them, should be capable of producing more inflorescences and flowers. The significant correlation between both inflorescence and flower number with plant size among male plants seems to confirm this. However, this correlation is lacking among female plants indicating that, for females, the number of inflorescences and flowers is independent of the size of the plant. Larger female plants do not produce more reproductive structures than smaller plants. This constraint is likely due to greater reproductive costs in females, not in the cost of flowers, but in the cost of seeds and fruit that must develop and mature during the most stressful months of the season. Because male plants are both more prevalent and produce more flowers per plant than female plants, pollen to ovule ratios will likely be high within a site. Thus, the need for precise synchronization of reproductive effort between male and female plants is allayed by the presence of a greater proportion of male plants enhanced by the larger number of inflorescences, a feature facilitated by an apparent lack of constraint on flower production in males. Extended periods of overlap in flowering between the sexes may also be achieved via female plants that may overcome the constraint on flower production. This is evident in the occasional prolongation of flowering and, thus, of stigma receptivity in some female plants. Relatively more robust female plants infrequently produce an additional series of fewer-flowered inflorescences distal to the initial series and separated from it by several nodes. Observations of fruit set indicate that these additional inflorescences do not occur as a response to insufficient pollination of the first series of inflorescences. Their production may simply be dependent on the energetics of the plant (Grime 1977; Chapin 1980). This continued production could be developmentally preprogrammed, but contingent upon resource availability. The delayed flowering of this second series could either facilitate pollination by later flowering pollen donors or insure, in cases in which the female plants flower early in the absence of pollen

donors, that at least some of the inflorescences are effectively pollinated.

When three assumptions are fulfilled, i.e., genetic determination of sex, lack of inbreeding, and lack of clonal growth, Fisher (1930) predicted a 1:1 zygotic sex ratio. The apparent absence of gender diphasy in *Smilax herbacea* implies probable heritable, genetic determination of sex rather than environmentally induced sex-switching (see Bawa and Beach 1981; Schlessman 1987). Further, the discovery of two hermaphrodite plants in 1995 (and their subsequent persistence) argues against sex determination by whole (X and Y) chromosomes as suggested by Mangaly (1968). As selfing, and therefore inbreeding, and clonal growth are also absent in the dioecious *S. herbacea,* Fisher's assumptions are fulfilled. Thus, we might suggest that the 2.3♂:1♀ sex ratio detected in this species arises post-zygotically in mature populations. With this assumption fulfilled, there are several possible explanations for the occurrence of the observed sex ratios. One likely explanation for male bias is the differential mortality of female plants. This possibility is supported by the conspicuous loss of seemingly robust females from among several populations between years. Our detection of a significant interaction between site and gender densities implies that site specific factors may also exert an influence on sex ratios. In dioecious species, environmental factors have been shown to influence male and female plants differently due to differences between the sexes in reproductive resource demands (Freeman et al. 1976; Lloyd and Bawa 1984; Niesenbaum 1992). The protraction of female reproductive life via fruit and seed production creates greater demands for resources in female plants than in males, and these demands occur during what are likely to be the more stressful summer months. Increased resource demand by females in concert with site-specific resource availability may explain increased female mortality, and this mortality may be responsible, in part, for variation in gender densities among sites.

Another possible explanation for the large male bias found for *Smilax herbacea,* viz., a difference in the flowering frequencies of males and females, is suggested by Allen and Antos (1993) who detected a significant male bias in their study of the dioecious Indian plum. In instances when females flower intermittently (i.e., do not always flower annually) or flower for the first time later in life than males, the presence of nonflowering females

among all nonflowering plants can be suspected if a strong association exists between an increase in nonflowering individuals and an increase in gender bias across populations (also see Lloyd and Webb 1977). In *S. herbacea*, we report a significant positive slope in the regression line when proportions of flowering male plants are plotted against proportions of nonflowering plants in all populations examined in both years ($r = 0.7701$, df = 15, P < 0.001). This suggests that females may flower less frequently than males. However, neither intermittent flowering nor first flowering of female plants was observed during the course of this study.

Although pollen limitation in flowering plants is thought to be a major factor affecting fruit and seed set (Burd 1994), this does not appear to be the case in *Smilax herbacea*. Despite the relatively low frequency of insect visitation to female flowers, resource, rather than pollen, limitation is indicated primarily by the fact that fruit set was not improved by hand pollination. The same level of fruit set in populations with proportionately fewer males vs. those with proportionately more males argues further against pollen limitation. One might posit that pollination opportunities will be greater in sites where male plant densities are higher, and that fruit set might be positively affected by these differences. However, the lack of a correlation between male plant densities and fruit set argues against this. Even if these opportunities are indeed present, plants appear unable to take advantage of them.

Mellitophily, saprocantharophily, and sapromyophily were borne out in observations of this species. Highly mobile bees, in addition to flies, are likely to be the major pollinators of *Smilax herbacea*. Halictid and andrenid bees collect pollen for brood rearing and may exhibit a host-parasite relationship with the odor of carrion-flower (Faegri and van der Pijl 1979). This odor is detectable at some distance, and is thus the likely primary attractant for most pollinators leading them to the pollen reward. First flowering males are likely to recruit a guild of pollinators that will encounter a pollen reward (as in dioecious *Lindera benzoin*; Carloni 1982). It is then likely that the pollen reward for these recruits comes to be associated with the carrion odor present in both sexes. This association thereby promotes effective pollination via the deceptive strategies of the female plants. The presence of many flowers in simultaneous anthesis in inflorescences of both male and female plants creates high levels of attractant odor

that is released into the environment by the tepals. Once in the vicinity of the flowers, visitors are adverted to conspicuous displays of pollen and, by deception in the case of females, to the greenish-white stigmas that appear to mimic pollen-laden anthers. This deception is fortified by the nearly identical appearance of the male and female inflorescences. In addition, the combination of easy accessibility, light to dull color, absence of nectar guides, strong odor, and presence in large inflorescences fits flowers characterized as those pollinated by small bees, flies, and beetles (Faegri and van der Pijl 1979).

Beetles are known to feed on pollen, and all beetle species observed in this study did so. Once a flower has opened, beetles often chew unopened anthers to get at the pollen reward, very likely shortening the reproductive life of the inflorescence. As noted above, the pollen is eaten rapidly by beetles, and this is one reason for males having pollen available for only a single day. The consumption of pollen was often so severe that inflorescences required bagging to ensure the availability of pollen for hand pollination experiments. The hard, relatively hairless bodies of beetles, and the fact that they seldom move among flowers or among plants, make them poor pollinators. In addition, the loss of pollen to beetle herbivory may be a greater cost than the benefit gained by beetle pollination.

The surviving hand pollination experiments resulted in a great deal of variation in fruit set. Unfortunately, many of the plants used in hand pollination experiments were destroyed by summer mowing. These included all 1995 intrapopulational attempts and, consequently, this resulted in low sample sizes. Significantly lower fruit set in 1995 was expected anyway due to widespread fungal infections promoted by the unusually wet conditions. The significant difference between intrapopulation and interpopulation crosses in the 1996 fruit set data (Table 4) might be a manifestation of inbreeding depression (Holsinger 1991, 1992). Fruit of *Smilax herbacea* is dispersed by songbirds (Martin et al. 1951). The large seeds probably are dropped principally within the site from which they were taken without passing through the bird's digestive system. Thus, the probability that genetically similar offspring will be dispersed within the same population may be higher than for long distance dispersal. Similarly, crosses between closely related individuals within populations may result in lower fruit set. Not surprisingly, fruit set was zero in all plants from

which bags were not removed; thus, there is no evidence for apomixis (with the exception of agamospermy, the possibility of which is not addressed herein).

In summary, we have detected sex ratios in dioecious *Smilax herbacea* populations that are strongly male biased. Although sex expression probably is determined genetically, sex ratios most likely are influenced by female mortality, which in turn may be influenced by site characteristics. Male plants, in addition to being more prevalent than female plants, produce more flowers in more inflorescences than female plants. Male plants show continual inflorescence production throughout the breeding season and exhibit greater variation in the number of inflorescences produced per plant than female plants. Female plants, on the other hand, are restricted in the number of inflorescences they are able to produce, presumably due to the costs of fruit and seed production. Although abundant pollen is available, continuous and severe pollen predation by beetles restricts pollen availability in some flowers in all populations. The fact that hand pollination failed to improve fruit set implies that female plants are resource rather than pollen limited.

With respect to its pollination syndrome, *Smilax herbacea* can be characterized as a generalist. Pollen movement in *S. herbacea* begins with the attraction of insect pollen vectors via the carrion-like odor of both staminate and pistillate flowers. Pollinators then are adverted to the conspicuous inflorescences of both male and female plants, where they are drawn to the pollen reward in male flowers and deceived by the anther-mimicking stigmas in the female flowers. Results of this study implicate halictid and andrenid bees and anthomyiid and stratiomyiid flies as major pollinators and species in three families of beetles as potential minor pollinators.

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